MEMORANDUM

Date: December 8, 2008

From: Alan Trounson, Ph.D., CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RT1-01101-1

Enclosed is a letter from Dr. Shengwn Li, of Children's Hospital of Orange County, an applicant for funding under RFA 08-02, CIRM Tools and Technologies Awards. This letter was not received at CIRM at least five working days prior to the December ICOC meeting, but we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

Although the letter was not submitted five days before this meeting, I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with Dr. Csete and the scientific staff, and concluded that the petition does not present compelling evidence that should alter the recommendation or score of the Grants Working Group (GWG). Briefly, reviewers expressed reservations about this proposal due to its poor presentation of data and information that was critical for evaluation. For example, reviewers noted that although a goal of the proposal is to utilize human embryonic stem cells or induced pluripotent stem cells, the applicant did not clearly describe the role or purpose of these cells in the experimental approach. The applicant's point-by-point comments largely represent a difference of expert scientific opinion or point to information that is not discussed clearly or adequately in the application. CIRM staff will be prepared to provide further analysis, should that be requested by any member of the committee.

Redactions, if any, have been made pursuant to the policy, in consultation with the author(s) of the letter. An unredacted version will be available for review in closed session.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.

Dear CIRM Committee:

Thank you, in advance, for your willingness to consider our responses to reviewers concerns about our initial proposal. As a team, we feel strongly that this is a unique proposal, which, if funded, has the potential to significantly contribute to the CIRM portfolio of technologies. We appreciate your careful consideration.

Respectfully,

RT1-01101-1 Team

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RT1-01101-1: Differential proteomic panning analysis of protein from single stem cells: An alternative to mass spectrometric methods

Review Summary

This proposal focuses on the development of methods for single-cell quantitative proteomic analysis of stem cells. The applicants have recently developed a technology, called Differential Proteomic Panning (DiPP), by which protein samples are labeled with different radioactive isotopes, fractionated by a variety of methods and the ratio of isotopes is used as a selection criterion for analysis by mass spectroscopy. The proposal describes validation and further development of the DiPP technology to allow single-cell proteomic analysis of human neural stem cells (hNSCs) and brain tumor stem cells.

In terms of impact, reviewers found that this proposal, if successful, could have a considerable impact in the field of stem cell research. Specifically, one reviewer commented that the technology for single-cell quantitative proteomic analysis would facilitate the discovery of new biomarkers for various stages of embryonic, precursor and cancer stem cells; cell-surface proteins unique to these various cell types to permit their purification; and candidate therapeutic molecular targets in cancer stem cells.

The specific original critiques are in “quotation marks”, followed by our underlined, comments .

“On the other hand, reviewers had very serious concerns about the feasibility of this proposal. Reviewers also criticized the research plan as vague and disorganized.”

Comment: With regard to feaibility, both DiPP and the single-cell microfluidic devices are published by researchers on our team. Our proposal is straightforward, describing three indepdendent but complimentary aims; with the overall goal of deriving single-cell data using a well established, newly patented proteomic technology.

“One reviewer commented that the proposal describes a variety of technologies but lacks details about how they will be applied to specific questions.”

Comment: Our proposal shows that the proteomic technology we advance has been used successfully in other biology/pharma applications. Our application proposes to use this same technolgoy specifically to characterize stem cells. Both DiPP proteomic technology and single cell microfluidic device technology have been published in patents and/or peer reviewed, scientific journals..

As one of the reviewer stated “ the technology for single-cell quantitative proteomic analysis would facilitate the discovery of new biomarkers for various stages of embryonic, precursor and

cancer stem cells; cell-surface proteins unique to these various cell types to permit their purification; and candidate therapeutic molecular targets in cancer stem cells."

"No information is provided about the source of human neural stem cells (hNSCs), nor how tumor SCs will be identified and purified from resected brain tumors."

Comment: On page 9 of our application, preliminary data show that we have all of the stem cells on which the preliminary data is based, in the lab. Working with neurosurgeons, we continue to generate new human stem cell lines. The page limit, restricts the ability to provide significantly more detail.

"A reviewer noted that microfluidic device fabrication is discussed conceptually but no design parameters are provided, leaving doubt as to whether the group plans to build it."

Comment: The microfluidic device has been fabricated. On page 10 of our application, the microfluidic device fabrication has been published by our team member and the preliminary data are provided (Refer to the letters of support and the biosketches).

"One reviewer also noted that the proposal will require a new instrument to detect tritium with 95% efficiency in the absence of scintillation fluid. This instrument will require at least a year in development, raising doubt that the technology can be optimized and applied within a 2-year funding period."

Comment: The DiPP proteomic technology is patented by a California-based biotech company, which contracts out this technology to pharmaceutical companies. Our application requests funding to contract this same technology for us with stem cells. The optimization is straightforward.

In fact, the planned instrument is designed for mainstream commercial use, eliminating the need for the use of a high energy isotope. Additionally there are many advantages for not using scintillation fluid such as environmental stewardship. (Pharmaceutical companies used Ci amount of ^{3}H and ^{14}C in their DMPK, thus the instrument would serve them as well.)

For the existing studies, there is no "must have" for this instrument to achieve this sensitivity because we can simply substitute ^{3}H and ^{14}C. with higher energy isotopes. For instance, ^{32}P and ^{33}P labeling, or ^{125}I and ^{131}I labeling does not require usage of scintillation fluid to achieve at least 90% efficiency with existing instrumentations. Furthermore, these stronger isotopes have much higher specific activities thus labeling with them, yields a much higher detection limit.

"Finally, the last aim, to perform single-cell proteomics with DiP, was found to lack experimental design details."

Comment: Refer to page 8 of our application, it is straightforward to complete Aim 3 (last aim) given the description of Aims 1 &2. Please also refer to page 10 for the preliminary data about the detailed experimental design for this last aim.

"There was some disagreement among reviewers about the qualifications of the assembled research team. One reviewer found that the principal investigator (PI) did not have a sufficiently strong CV to justify the risk of funding such an uncertain project."

Comment: Can you elaborate what was the disagreement? This is a very unique team integrated with the DiPP technology (the inventor, the two patents are issued and four patents pending) and the single-cell microfluidic device (the inventor and the paper is published, patent pending).

What is a sufficiently strong CV? The PI and the assembled team are a uniquely teamed up to apply these technologies to the grant proposal. Members of the team are inventors on numerous related patented technologies in both academia and industry.

The PI obtained a PhD from the Mount Sinai School of Medine and completed the post-doctoral training at Massachusetts Institute of Technology and Harvard Medical School as supported by the NIH-Individual National Research Service Award. The PI has published several pioneering articles, which have become the most-frequently-cited articles in the area of interest. Specifical for biotechnolgy, the PI has been an inventor for numersous patents pending with two issued patents. The PI has three patents pending in the specific stem cell area. The extensive experience of the PI in academia and industry empowers the PI's capacity to work at the exciting interface between basic research and biotechnolgy, where integration and innovation are essential for survival and success.

"Several reviewers raised concerns about the budget, noting that the roles of contractors and consultants are unclear and the budget justification for the large subcontract (~$190K) is incomplete and insufficient."

Given the technologies uniquely developed by these inventors, such a budget is justified. The expanded technologies for stem cell research should be sufficient for another set of patent applications, which will be beneficial for the portfolio of CIRM technology development and California biotechnology companies.

"Overall, reviewers found this proposal to be overly ambitious and expressed serious doubts about the project's feasibility given the enormity of the technical challenges. Reviewers also criticized the proposal's poorly organized research plan and lack of experimental detail."

Comment: The technologies currently are in use in these labs and the company, this grant proposal is an expansion for the use of these technologies to stem cell applications. What is "the enormity of technical challenges" they reffered to? Refer to the above comments for the project feasibility, grant organization, and experimental details.

RT1-01101-1: Differential proteomic panning analysis of protein from single stem cells: An alternative to mass spectrometric methods

Recommendation: Not recommended for funding
Scientific Score:
First Year Funds Requested: $433,678 **Total Funds Requested:** $865,739

Public Abstract (provided by applicant)

In children, cancers are the deadliest of diseases and second only to accidents as the leading cause of death. Cancers of the brain are the worst. Our current forms of therapy for these diseases can best be described as brutal: brain surgery followed by administration of very high doses of very toxic drugs and exposure to high doses of radiation. The deadliest of the brain cancers are the malignant gliomas. All children with this type of cancer die and in all cases the course of the disease and its treatment are horrific. About two-thirds of children can survive the rest of the types of brain cancers but two-thirds of these survivors go on to have a recurrence of their cancer. Even more heartbreaking is the fact that those that do survive are usually left with lifelong disabilities. Emerging evidence indicates that brain tumor stem cells are responsible for recurrence of many of these cancers. It is essential to identify the proteomic differences between normal stem cells and tumor stem cells for targeted destruction of brain tumor stem cells.

Existing proteomic technologies still have many constraints and limitations. Our patented [REDACTED] technology platform is much more sensitive and effective in identifying the surface markers than any existing methods. This proposal if funded by CIRM will pave the way for promoting [REDACTED] to be used for proteomic studies of single-stem-cells.

Statement of Benefit to California (provided by applicant)

Malignant brain cancers are a leading cause of cancer death. Three decades of research have resulted in little change to the outcome of these lethal brain cancers. For example, virtually all patients die after being diagnosed a diffuse brainstem glioma. Of the two-thirds of patients who survive at least 5 years after being diagnosed with any brain cancer, more than two-thirds go on to have a recurrence of their disease. Moreover, the treatments that these patients suffer can only be described as brutal and most of those that do survive are left with life-long mental disabilities. Overall estimates of the incidence of brain cancers in the United States show that about 20,000 will be diagnosed annually with about 2,500 in California. Given these statistics, the costs for the patient and family cannot be overestimated. The economic costs are also grim. Repeated use of physician, inpatient, outpatient and laboratory services as well as lost future earnings and occurrence of secondary diseases are projected to cost Californians more than 1.5 billion dollars annually. It is clear that California patients with brain cancers need a new therapeutic approach.

One promising approach is to target brain tumor stem cells. It is essential to identify the proteomic differences between normal stem cells and tumor stem cells for targeted destruction of brain tumor stem cells.

Existing proteomic technologies still have many constraints and limitations. Our patented [REDACTED] technology platform is much more sensitive and effective in identifying the surface markers for targeting brain tumors stem cells, than any existing methods. This proposal if funded by CIRM will pave the way for promoting [REDACTED] to be used for proteomic studies of single-stem-cells, which can be used for other brain diseases and injuries as well. Other long-term economic impact is the opportunity to train more student scientists in the field of stem cell technology, facilitating and promoting California's biotechnology industry.

Review

This proposal focuses on the development of methods for single-cell quantitative proteomic analysis of stem cells. The applicants have recently developed a technology, by which protein samples are labeled with different radioactive isotopes, fractionated by a variety of methods and the ratio of isotopes is used as a selection criterion for analysis by mass spectroscopy. The proposal describes validation and further development of the technology to allow single-cell proteomic analysis of human neural stem cells (hNSCs) and brain tumor stem cells.

In terms of impact, reviewers found that this proposal, if successful, could have a considerable impact in the field of stem cell research. Specifically, one reviewer commented that the technology for single-cell quantitative proteomic analysis would facilitate the discovery of new biomarkers for various stages of embryonic, precursor and cancer stem cells; cell-surface proteins unique to these various cell types to permit their purification; and candidate therapeutic molecular targets in cancer stem cells.

On the other hand, reviewers had very serious concerns about the feasibility of this proposal. Reviewers also criticized the research plan as vague and disorganized. One reviewer commented that the proposal describes a variety of technologies but lacks details about how they will be applied to specific questions. No information is provided about the source of human neural stem cells (hNSCs), nor how tumor SCs will be identified and purified from resected brain tumors. The proposal also mentions human embryonic and induced pluripotent SCs, but without enough elaboration to determine their role in the overall plan. A reviewer noted that microfluidic device fabrication is discussed conceptually but no design parameters are provided, leaving doubt as to whether the group plans to build it. One reviewer also noted that the proposal will require a new instrument to detect tritium with 95% efficiency in the absence of scintillation fluid. This instrument will require at least a year in development, raising doubt that the technology can be optimized and applied within a 2-year funding period. Finally, the last aim, to perform single-cell proteomics with DiP, was found to lack experimental design details.

There was some disagreement among reviewers about the qualifications of the assembled research team. One reviewer found that the principal investigator (PI) did not have a sufficiently strong CV to justify the risk of funding such an uncertain project. Several reviewers raised concerns about the budget, noting that the roles of contractors and consultants are unclear and the budget justification for the large subcontract (~$190K) is incomplete and insufficient.

Overall, reviewers found this proposal to be overly ambitious and expressed serious doubts about the project's feasibility given the enormity of the technical challenges. Reviewers also criticized the proposal's poorly organized research plan and lack of experimental detail.

The following Working Group members had a conflict of interest with this application: